

# Stress-Related Mediators Stimulate Vascular Endothelial Growth Factor Secretion by Two Ovarian Cancer Cell Lines

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## ABSTRACT

**Purpose:** Stress has long been believed to influence carcinogenesis, but little is known about physiological mechanisms that may underlie these effects. We have recently observed lower levels of vascular endothelial growth factor (VEGF) in ovarian cancer patients with greater social support, whereas higher VEGF was found in patients with greater distress. The goal of this study was to examine possible mechanisms underlying these relationships.

**Experimental Design:** The effects of stress-related mediators including norepinephrine (NE), epinephrine, isoproterenol (a nonspecific  $\beta$ -adrenergic agonist), and cortisol on the production of VEGF by the ovarian cell lines SKOV3 and EG were investigated.

**Results:** NE and isoproterenol significantly enhanced VEGF production by SKOV3 cells, and all three of the adrenergic agonists enhanced VEGF production by EG cells. These effects were blocked by the  $\beta$  antagonist pro-

pranolol, supporting a role for  $\beta$ -adrenergic receptors in these effects. Reverse transcriptase-PCR studies indicated constitutive expression of  $\beta$ -1 and  $\beta$ -2 adrenergic receptors on both cell lines. Effects of cortisol on VEGF production varied according to the specific cell line and dose, with stimulating effects on SKOV3 at pharmacologic doses (1000 nM) and on EG at physiological stress level doses (10 nM), and inhibitory effects on EG at pharmacologic doses. Although priming with cortisol blunted NE-induced VEGF production from both cell lines at 3 h, significant increases in VEGF were still seen. Priming with cortisol enhanced isoproterenol-induced VEGF production from SKOV3.

**Conclusion:** These findings provide the first experimental evidence of a pathway by which biobehavioral stress mediators could directly contribute to the progression of ovarian tumors.

## INTRODUCTION

There is a growing literature reporting effects of stress on the immune system in cancer patients, including studies citing lower levels of NK<sup>2</sup> cell activity, diminished response of NK cells to recombinant IFN $\gamma$ , and decreased proliferative response to mitogens among patients with high levels of stress (1–7). Surgical stress and other experimental stressors have been associated with suppressed NK cell activity and increased tumor progression in an experimental model of breast cancer (2). However, little is known about other mechanisms by which biobehavioral processes may influence growth and progression of cancer. Angiogenesis is a key process in the growth of most solid tumors and their metastatic spread (8), and involves recruitment of nearby blood vessels to permeate the tumor (8, 9). Angiogenesis is regulated by a number of factors including VEGF, a homodimeric  $M_r$  32,000–42,000 heparin-binding glycoprotein thought to be one of the most important proangiogenic cytokines in cancer (10). VEGF is primarily produced by tumor cells, endothelial cells, and platelets (11–14), and works by stimulating endothelial cells in microvessels to proliferate, migrate, and alter their pattern of gene expression. It also makes cells hyperpermeable, resulting in conditions that favor angiogenesis in the extracellular matrix (15). VEGF appears to play a key role in the pathogenesis of ovarian cancer and has been seen as a prognostic indicator in that higher VEGF levels are associated with metastatic disease (16) and poorer survival (17, 18). Tumor indicators of angiogenesis, assessed by microvessel density counts and by VEGF expression have also been reported as prognostic indicators in ovarian cancer (19). We have observed

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<sup>2</sup> The abbreviations used are: NK, natural killer; VEGF, vascular endothelial growth factor; NE, norepinephrine; E, epinephrine; RT-PCR, reverse transcriptase-PCR;  $\beta$ 1AR,  $\beta$  1 adrenergic receptor;  $\beta$ 2AR,  $\beta$  2 adrenergic receptor.

recently that among ovarian cancer patients, lower levels of VEGF are found in patients with greater social support, and higher VEGF is found in patients with greater distress (20). Little, however, is known about possible mechanisms that may underlie these relationships in ovarian cancer.

VEGF production is regulated by a number of hormones and cytokines (21–23). VEGF can be induced by NE, one of the main neurotransmitters in the sympathetic nervous system (24–26), and a hormone that is also produced by the adrenal medulla during acute and chronic stress. NE has been shown to up-regulate VEGF in brown adipose tissue (24, 25), but there have been no reports of a direct effect of NE on VEGF production by cancer cells. Similarly, it is not known whether the sympathetic neuroeffector E, which is released from the adrenal medulla during stress, might affect production of VEGF by tumor cells.  $\beta$ -Adrenergic receptors, which mediate many sympathetic functions of E and NE, have been documented on mammary tumors (27), and their activation has been related to mammary tumor growth (28). To date, to the best of our knowledge, there has been no documentation of  $\beta$ -adrenergic receptors on ovarian tumor cells. It is also not known whether NE or other catecholamines can increase VEGF production by ovarian cancer cells.

Another stress hormone that influences VEGF is cortisol, a glucocorticoid hormone secreted by the adrenal cortex and also elevated in depression (29). Dexamethasone, a synthetic glucocorticoid, has been shown to down-regulate VEGF in glioma cells, although the effect is markedly decreased in hypoxic conditions, as occur in rapidly growing tumors (30). Very low levels of dexamethasone ( $10^{-5}$ – $10^{-7}$  M) have been found to stimulate tumor growth, suggesting a bimodal effect of this hormone on tumor growth (31). Cortisol is also known to act synergistically with adrenergic cellular mechanisms; for example, it potentiates adrenergically induced increases in cyclic AMP in tumor cells, thus enhancing growth (32). Whereas stress has been associated with high levels of cortisol and catecholamines, social support has been associated with lower levels of catecholamines and cortisol in several studies (33, 34), and stress reduction has been associated with a reduction in levels of these hormones (35, 36).

On the basis of the relationships outlined above, we hypothesized that mediators of stress such as NE and E would directly stimulate production of VEGF by ovarian cancer cells, whereas effects of cortisol on VEGF production would be dependent on dose. The experiments in this study were undertaken to examine possible mechanisms underlying relationships between stress-related hormones and VEGF production in two ovarian cancer cell lines.

## MATERIALS AND METHODS

**Cell Culture.** NE, E, isoproterenol, and cortisol for *in vitro* use were obtained from Sigma Aldrich (St. Louis, MO). The derivation and source of the established ovarian cancer cell lines SKOV3 and EG have been reported previously (37). These cells were maintained and propagated *in vitro* by serial passage in RPMI 1640 supplemented with 15% fetal bovine serum and 0.1% gentamicin sulfate (Gemini Bioproducts; Calabasas, CA). All of the cell lines are routinely screened for *Mycoplasma*

species (GenProbe detection kit; Fisher, Itasca, IL). All of the experiments were performed with 70–80% confluent cultures. Cells were seeded in 12-well plates, with  $2.5 \times 10^5$  cells/well cultured for 1 day, and then treated with isoproterenol, E, or NE at 0, 0.1, 1, and 10  $\mu$ M or with cortisol at 0, 1, 10, 100, and 1000 nM. In our first set of experiments, supernatants were removed at 12 and 24 h, centrifuged, and frozen at  $-80^\circ\text{C}$  until assay. To simulate effects of joint presence of stress hormones within the body, costimulation experiments were performed with isoproterenol or NE and cortisol. In these experiments cells were primed with 10 nM cortisol at the time of seeding, and then subsequently stimulated 24 h later with (0, 0.1, 1, and 10  $\mu$ M) isoproterenol or NE, and the supernatants were harvested as described above. Experiments were subsequently replicated at 3 and 6 h to determine earlier effects of specific stress hormones on VEGF. For blocking experiments, propranolol 1  $\mu$ M was added to the cell cultures 1 h before adding 0, 1, or 10  $\mu$ M of NE, E, or isoproterenol. Supernatants were removed at the time of maximal VEGF production for each mediator and for each cell line. Each experiment was repeated at least three times in duplicate with unstimulated cells and media controls except for costimulation experiments, which were performed two to six times in duplicate with the same controls.

**RT-PCR Analysis.** Total RNA was isolated from unstimulated cells and cells stimulated with Isoproterenol using the RNeasy minikit (Qiagen). The RNA was quantified with spectrophotometry at 260 and 280 nm as described previously (38). The RNA was DNase digested and reverse transcribed as described previously (38). The resulting cDNA was amplified by PCR in Gene Amp 10 $\times$  PCR buffer (Perkin-Elmer, Branchburg, NJ) with 20 pmol of gene specific 3' and 5' primers, 2 units of TaqDNA polymerase in a total volume of 50  $\mu$ l. The following primers were used for  $\beta$ 1AR: forward, 5' – TTTGGGGAAGGGATGGGAGAG – 3'; reverse, 5' – CCTGGTGGGGGAAAAAATC – 3'; and for  $\beta$ 2AR: forward, 5' – CATGTCTCTCATCGTCCTGGCCA-3'; reverse, 5' – CACGATGGAAGAGGCAATGGCA-3'. PCR conditions for  $\beta$ 1AR included initial incubation at  $94^\circ\text{C}$  for 12 min followed by 38 cycles with  $94^\circ\text{C}$  for 1 min,  $60^\circ\text{C}$  for 2.5 min, and  $72^\circ$  for 1 min. PCR conditions for  $\beta$ 2AR were: initial incubation at  $94^\circ\text{C}$  for 12 min followed by 38 cycles with  $94^\circ$  for 1 min and  $72^\circ$  for 3.5 min, and a final incubation at  $72^\circ\text{C}$  for 5 min. After RT-PCR, the reaction products were separated by electrophoresis on a 1% agarose gel. The gels were stained with ethidium bromide to visualize the PCR product size. To control for variance in loading and in PCR, samples were compared with glyceraldehyde-3-phosphate dehydrogenase PCR products.

**VEGF.** Detection of VEGF present in supernatants was performed by an ELISA using standard kits (R&D Diagnostics, Minneapolis, MN; VEGF Quantikine kit), with measurements performed according to the manufacturer's instructions and results interpolated from the standard reference curve provided with the kit. The minimum detectable level of VEGF is <5.0 pg/ml.

**Statistical Analysis.** The SPSS version 11.0 (SPSS Inc., Chicago, IL) was used for all of the analyses. Data were analyzed by repeated measures ANOVAs, with time as the within factor and dose as the between factor to determine whether there was a statistically significant main effect for dose. Significant

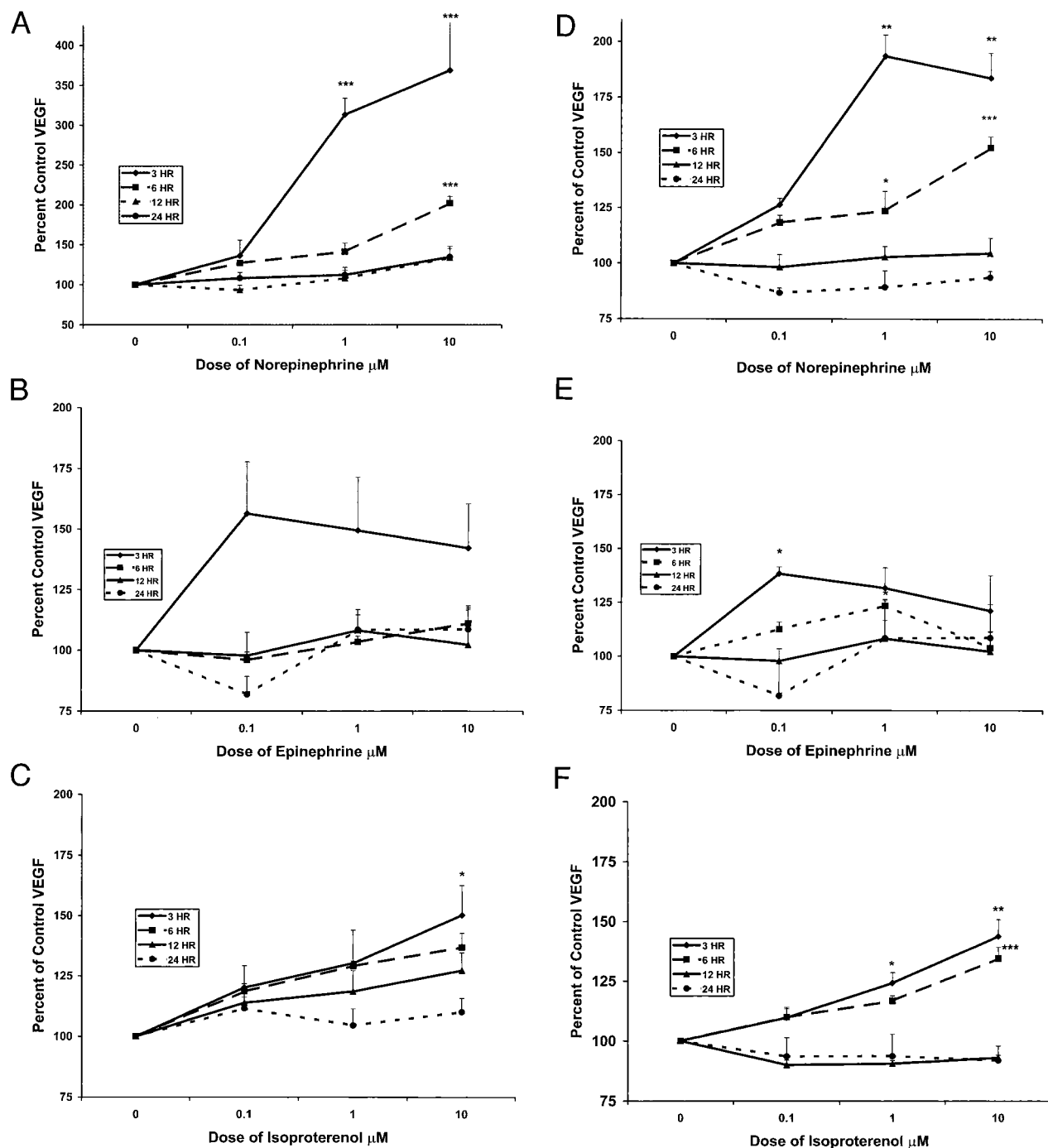


Fig. 1 VEGF production by SKOV3 cells incubated with 0, .1, 1, 10, and 100  $\mu\text{M}$  NE (A), E (B), isoproterenol (C), and EG cells incubated with these doses of NE (D), E (E), and isoproterenol (F). In experiment 1, supernatants were harvested at 12 and 24 h; in experiment 2, supernatants were harvested at 3 and 6 h. For ease of comparison all time points are shown together in the figures. All VEGF levels (pg/ml) were analyzed by ELISA. Statistical analyses were done within experiment 1 or experiment 2. Data are represented as percentage of the control (media only) well, which was set to 100% in each experimental series. All data points represent the mean of at least three experiments performed in duplicate. All panels have 200% of control as the top of the Y-axis except for A, which extended to 400%. Significant overall repeated measures ANOVAs were followed by Dunnett's 2-sided *t* tests. \* indicates significant differences as compared with the control wells (with media alone added to the cells) when the *P* was  $<0.05$ ; \*\*, *P*  $\leq 0.01$ ; \*\*\*, *P*  $\leq 0.001$ . Bars,  $\pm$ SE.

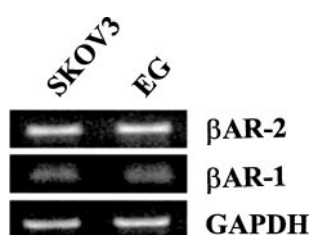


Fig. 2 RT-PCR analysis of  $\beta$ -adrenergic receptors ( $\beta$ 1AR and  $\beta$ 2AR) in the ovarian cancer cell lines SKOV3 and EG.

main effects were followed up with Dunnett's tests to control the experimentwise error rate. All of the statistics reported in text are from Dunnett's tests and represent comparisons to the controls. In experiment 1, supernatants were harvested at 12 and 24 h; in experiment 2, supernatants were harvested at 3 and 6 h. Statistical analyses were done separately within experiment 1 and experiment 2. For clarity of comparison among cell lines, time points, and stress hormones, figures are represented as percentage of control values with the control well set at 100%.

## RESULTS

### Adrenergic Stimulation of Ovarian Cancer Cell Lines.

SKOV3 and EG cell lines were stimulated with the specified doses of NE, E, and isoproterenol, and the supernatants were assayed for VEGF production as shown in Fig. 1, A–F. The mean basal level of VEGF production by SKOV3 cells at 3 h with no stimulation was 75.72 pg/ml ( $SD \pm 52.83$ ); the mean basal production of VEGF by EG cells at 3 h was 220.05 pg/ml ( $SD \pm 67.84$ ) with no stimulation. For both cell lines, NE elicited the most robust VEGF response; maximum increases in VEGF occurred at 3 h of incubation. As illustrated in Fig. 1A, incubation of SKOV3 cells with 1  $\mu$ M NE for 3 h produced an approximately 2-fold increase in VEGF production ( $P = 0.001$ ), and 10  $\mu$ M NE produced a 2.7 fold increase in VEGF ( $P < 0.001$ ) as compared with the control. At 6 h, 10  $\mu$ M NE produced a significant increase in VEGF ( $P < 0.001$ ) over the control, and there was a trend to a significant increase for the 1  $\mu$ M dose ( $P = 0.058$ ). For E, the maximum VEGF increase (156% of control) occurred at 3 h with 0.1  $\mu$ M E and then plateaued at higher doses. (Fig. 1B) However, the overall VEGF induction by E in the 3- and 6-h model did not differ significantly from the control values. For isoproterenol (Fig. 1C), the maximum VEGF increase observed at 3 h of incubation with 10  $\mu$ M isoproterenol was 150% of the control well ( $P = 0.017$ ). Induction of VEGF at other time points from SKOV3 by isoproterenol was not significant (Fig. 1C).<sup>3</sup>

To determine the effects of stress hormones on a different epithelial ovarian cancer cell line, we used EG cells, and similar patterns of VEGF stimulation were observed (Fig. 1, D–F). The maximum level of VEGF induced in EG cells by NE was 194% of control, observed at 3 h after incubation with 1  $\mu$ M NE (Fig. 1D). At 3 and at 6 h, both 1 and 10  $\mu$ M of NE produced significant

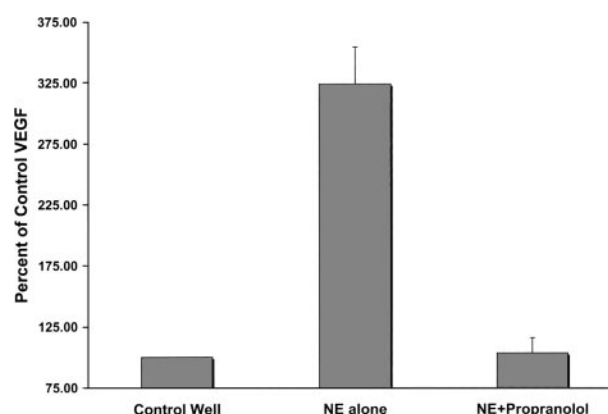


Fig. 3. Representative blocking experiment (B). SKOV3 cells were incubated for 3 h with 0  $\mu$ M NE (control well), 10  $\mu$ M NE (center), or 1  $\mu$ M propranolol one h before incubation with 10  $\mu$ M NE (right). Data are represented as percentage of the control (media only) well, which was set to 100%. All data points represent the mean of at least three experiments performed in duplicate. Bars,  $\pm$ SE.

increases in VEGF (3 h:  $P = 0.005$ ,  $P = 0.007$ , respectively; 6 h:  $P = 0.03$ ,  $P < 0.001$ , respectively). E (0.1  $\mu$ M) significantly induced VEGF secretion at both 3 ( $P = 0.028$ ) and 6 h ( $P = 0.03$ ). For both cell lines, the maximum E stimulation was induced by 0.1  $\mu$ M rather than by the higher doses of NE, which were necessary to induce maximum production of VEGF (Fig. 1E). Isoproterenol produced significant increases in VEGF at 3 and 6 h (3 h: 1  $\mu$ M,  $P = 0.02$ ; 10  $\mu$ M,  $P = 0.002$ ; 6 h: 1  $\mu$ M,  $P = 0.053$ ; 10  $\mu$ M,  $P = 0.001$ ; Fig. 1F). Induction of VEGF at 12 and 24 h was not significant for any mediator nor for either cell line.

**Assessment of  $\beta$ -Adrenergic Receptors and Blocking Experiments.** RT-PCR analysis revealed that both  $\beta$ -1 and  $\beta$ -2 adrenergic receptors were expressed by both SKOV3 and EG cell lines (Fig. 2). To test whether VEGF stimulation could be mediated through  $\beta$ -adrenergic receptors, both cell lines were treated with a nonspecific  $\beta$  antagonist, propranolol 1  $\mu$ M, for 1 h before stimulation with isoproterenol, NE, or E at the time points of maximum VEGF release. Adrenergically induced VEGF release was completely blocked by propranolol pretreatment (Fig. 3).

### Stimulation with Cortisol and Costimulation Experiments.

Both ovarian cancer cell lines were treated with different doses of cortisol, and results are shown in Fig. 4, A and B. For SKOV3, a significant decrease in VEGF was seen after incubation with 1 nM cortisol ( $P = 0.017$ ) at 3 h, whereas a small but statistically significant increase in VEGF production was seen after incubation with 1000 nM cortisol ( $P = 0.012$ ; Fig. 4A). The latter dose is approximately equivalent to pharmacological levels of dexamethasone. At 6 h, a similar but nonsignificant trend was seen with the 1000 nM dose of cortisol. In contrast, at 12 and 24 h, both 1000 nM doses caused suppression in VEGF, but these changes did not reach significance. For EG, 6 h of incubation with 10 nM cortisol (simulating physiological stress levels) induced a significant increase in VEGF ( $P = 0.046$ ; Fig. 4B). However, with increasing cortisol concentrations, VEGF declined, showing a significant decrease after incubation with 1000 nM cortisol at 6 h ( $P = 0.037$ ). At both 12

<sup>3</sup> The main effect for dose in the overall 3–6 h repeated measures ANOVA was marginally significant at 0.073.

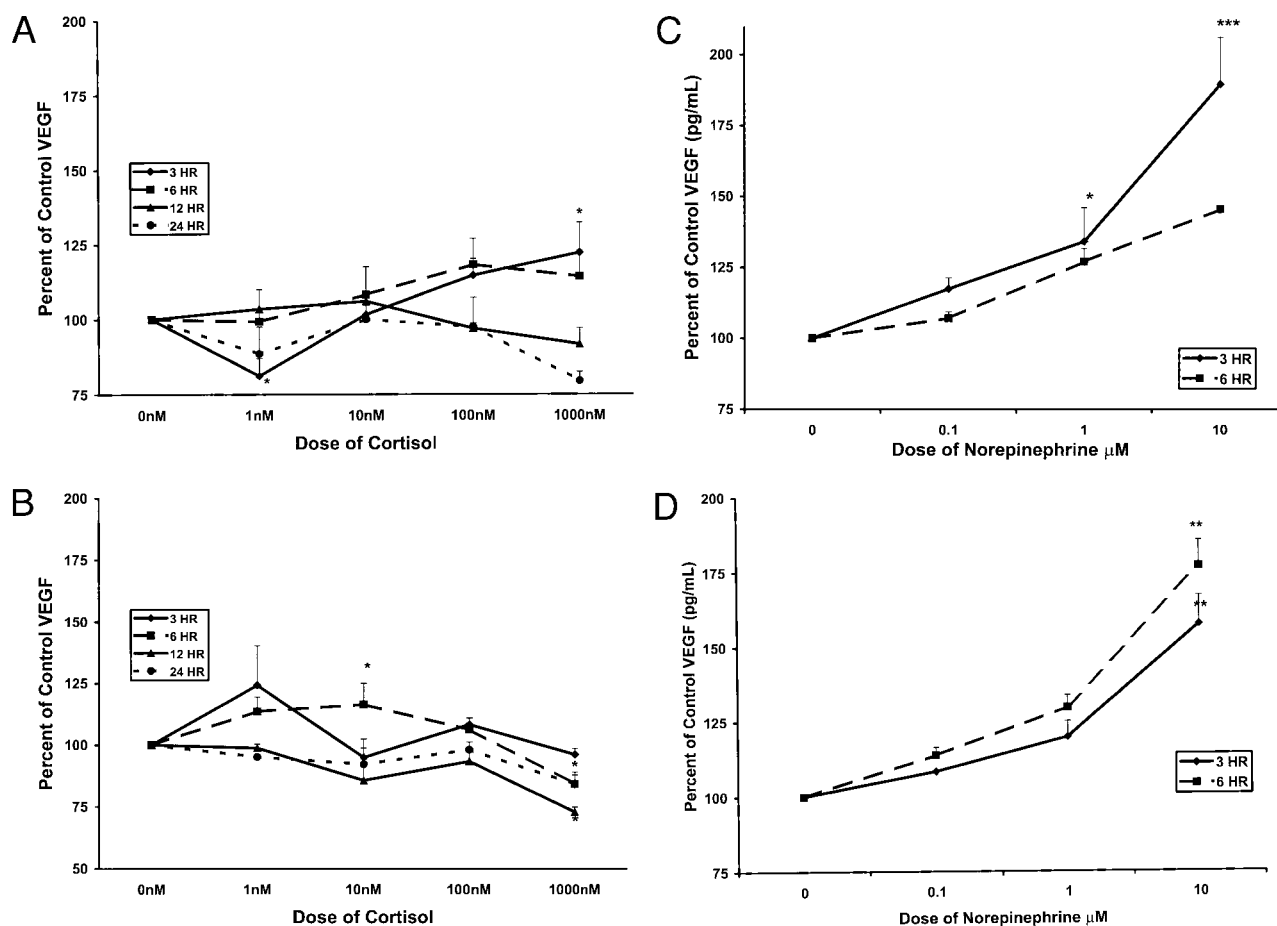


Fig. 4 VEGF production by SKOV3 (A) and EG cells (B) incubated with 0, 1, 10, 100, and 1000 nM cortisol. In experiment 1, supernatants were harvested at 12 and 24 h; in experiment 2, supernatants were harvested at 3 and 6 h. Statistical analyses were done within each experiment. Data are represented as percentage of the control (media only) well, which was set to 100% in each experimental series. All data points represent the mean of at least three experiments performed in duplicate. VEGF production by SKOV3 cells (D) and EG cells (E) incubated with 0, 0.1, 1, and 10 μM NE after priming with 10 nM cortisol. Supernatants were harvested at 3 and 6 h, and VEGF levels were analyzed by ELISA. Data are means of two to three experiments with all experiments done with duplicate wells. Data are represented as percentage of the control well (cells incubated in the absence of NE), set at 100%. Significant increases when compared with control are indicated by \*, for  $P < 0.05$ ; \*\*,  $P \leq .01$ ; \*\*\*,  $P \leq .001$ . Bars,  $\pm$ SE.

and 24 h, all doses of cortisol produced decreases in VEGF production in EG cells, but these did not reach significance.

Next we assessed the influence of cortisol on catecholamine-mediated VEGF stimulation of both ovarian cancer cell lines. These experiments were performed with NE and isoproterenol, because these mediators had produced consistent stimulation in both cell lines. Results of costimulation experiments suggested that effects of priming cells with 10 nM cortisol varied according to the adrenergic agent, the time point, and the specific cell line. For both cell lines, the NE effect on VEGF stimulation was generally dominant even when the cells were pretreated with cortisol (Fig. 4, C and D).

Specifically, in the series of experiments cited above, 3 h of incubation of SKOV3 with 10 μM NE alone induced VEGF production that was 370% of the control (Fig. 1A); in experiments including priming with cortisol and 3-h incubation with 10 μM NE, VEGF levels dropped to 190% of the control (Fig.

4C).<sup>4</sup> The NE-induced VEGF secretion after cortisol priming was still significant at 3 h as compared with the control wells (1 μM,  $P = 0.03$ ; 10 μM,  $P = 0.001$ ). In EG cells, effects of cortisol priming depended on the incubation time. In the series of experiments involving NE stimulation of VEGF, after 3 h of incubation with NE alone, maximum VEGF secretion was 183% of control (Fig. 1D); in the priming experiments, this dropped to 157% of control at 3 h (Fig. 4D). In contrast, at 6 h, priming with cortisol appeared to enhance VEGF. After 6 h of incubation with 10 μM NE alone, mean VEGF secretion was 152% of control; in the priming experiments, mean VEGF secretion

<sup>4</sup> These values were not compared statistically because they are the result of different series of experiments. Thus, inferences from these comparisons with priming experiments should be considered as preliminary.



increased to 177% of control. The VEGF levels after cortisol priming in EG cells were significant at both 3 and 6 h as compared with the control wells ( $P = 0.006$  at both time points).

With isoproterenol, priming with cortisol appeared to enhance VEGF production in SKOV3 cells. In the experiments conducted in the absence of cortisol, maximum isoproterenol-induced production of VEGF was 150% of control at 3 h and 137% of control at 6 h (Fig. 1C). After cortisol priming these values rose to 168% of control and 148% of control, respectively (data not shown). The VEGF secretion in SKOV3 cells after cortisol priming was significantly elevated as compared with the control wells after 3 h (1  $\mu\text{M}$ ,  $P = 0.009$ ; 10  $\mu\text{M}$ ,  $P < 0.001$ ), but not at other time points.<sup>5</sup> After cortisol priming, isoproterenol stimulation in EG produced significant increases of VEGF over the control at both 3 and 6 h (3 h: 10  $\mu\text{M}$ ,  $P = 0.031$ ; 6 h: 10  $\mu\text{M}$ ,  $P = 0.048$ ). However, with isoproterenol, VEGF secretion at 3 h but not at 6 h was lower than from nonprimed conditions.

## DISCUSSION

These results indicate that stress-related mediators (NE, E, and isoproterenol) can directly enhance the production of the proangiogenic cytokine VEGF from two ovarian cancer cell lines. These effects are mediated through  $\beta$ -adrenergic receptors because propranolol, a  $\beta_1$  and  $\beta_2$  receptor antagonist, eliminated the VEGF stimulation induced by these adrenergic agents. Furthermore, the presence of  $\beta_1$  and  $\beta_2$  adrenergic receptors in both cell lines was demonstrated by RT-PCR. Because angiogenesis is an important component of the estrous cycle, the regulation of ovarian angiogenesis by hormones has been suggested previously (23). However, to the best of our knowledge, this is the first report demonstrating direct regulation of a proangiogenic cytokine in ovarian cancer cells by stress-related hormones.

The effective dosages of stress-related hormones ( $\leq 10 \mu\text{M}$ ) were within the levels that would be produced in the body from stress-related catecholamine secretion. VEGF induction in both cell lines was more robust at 3 h than at later time points. This timing is consistent with the relatively rapid course of action of these adrenergic agents (39). Moreover, effects of NE were more pronounced than effects of E in both cell lines, although E did have significant effects on EG. Reasons for this difference in the effects of NE and E are not totally clear at this time. We have shown constitutive expression of both  $\beta_1$  and  $\beta_2$  receptors on both ovarian cancer cell lines.  $\beta_1$  receptors are known to be more sensitive to NE than to E, whereas  $\beta_2$  receptors are more sensitive to E (39). Whether the differential response of ovarian cancer cells is because of differences in relative ratio of  $\beta$ -receptor subtypes is not known and is the subject of ongoing research in our laboratory.

Moreover, it should be noted that our findings with E stimulation of SKOV3 represent the results of a conservative statistical analysis, and the lack of a significant main effect for dose in the overall test is likely because of the absence of effects

at 6 h. If the 3-h stimulation of SKOV3 by E is examined by itself in comparison with control values using Dunnett's test, VEGF induction by each dose of E is significant, ranging from  $P = 0.006$  at 0.1  $\mu\text{M}$  to  $P = 0.032$  at 10  $\mu\text{M}$ . Thus, future examination of E induction of VEGF at 3 h is likely to be productive.

Our findings are consistent with a previous report of increased VEGF expression in brown adipose tissue from rats in a cold-exposure stress paradigm (25). This increased VEGF expression was activated by sympathetic nerves, abolished by surgical sympathetic denervation, and mimicked by administration of NE or a  $\beta$ -adrenergic agonist (24, 25). Endogenous NE has also been shown to increase VEGF expression in a dose- and time-dependent manner in adipocytes, an effect that was abolished by propranolol. NE also up-regulated transcription of the VEGF gene in adipocytes (26).

Effects of cortisol differed according to the dose of cortisol and cell line. As hypothesized, in EG cells at 6 h, cortisol at physiological stress levels (10 nM) increased VEGF secretion, whereas cortisol levels that were closer to pharmacologic concentrations (1000 nM) induced significant reductions in VEGF. Similar nonsignificant patterns were observed at all of the time points in EG cells at 1000 nM. This is consistent with the demonstration of an inhibitory effect of dexamethasone on VEGF gene expression by rat glioma cells (30). In contrast, with SKOV3 cells a pattern opposite to that hypothesized emerged. Low cortisol levels (1 nM) decreased VEGF secretion, whereas pharmacologic concentrations increased VEGF secretion at 3 h. This suggests possible differences in receptors or in downstream activation patterns between the two cell lines. In glioma cells it has been noted that the hypoxia-induced up-regulation of VEGF was more pronounced than the effect of the dexamethasone (30), suggesting the importance of repeating the present experiments in normoxic and hypoxic conditions to clarify cortisol effects in both.

Because stress states often involve elevations in cortisol and catecholamines, we used a costimulation paradigm to simulate conditions that might be seen in the body under acute or chronic stress. The enhancement of VEGF production by SKOV3 cells after costimulation with cortisol and isoproterenol, and after costimulation of EG with cortisol and NE at 6 h is consistent with reports that glucocorticoids increase  $\beta$ -adrenergic receptor density in pulmonary adenocarcinoma cells (32, 40) and potentiate adrenergically induced increases in cyclic AMP in these tumor cells (32). One report has also indicated that  $\beta_2$  adrenoreceptors were increased in number and responsiveness to catecholamines as a result of chronic stress-induced elevations in plasma cortisol (41). In contrast, cortisol appeared to blunt effects of NE on both SKOV3 and EG at 3 h. Mechanisms underlying these differences are still unclear and are the target of ongoing research.

These findings demonstrate that stress hormones can stimulate production of a potent proangiogenic factor by ovarian cancer cells and provide the first experimental evidence of a pathway by which a biobehavioral stress state could directly influence the progression of a malignancy. Social support has been associated with lower tonic levels of stress hormones including E, NE, and cortisol (34, 42–44). Stress reduction has also been associated with decreased levels of these neuroendo-

<sup>5</sup> The main effect for dose in the overall repeated measures ANOVA was marginally significant at 0.057.

crine hormones (35, 36). Thus, the present results suggest pathways that may underlie our previous finding of a relationship between greater social support and lower levels of serum VEGF in ovarian cancer patients (20). Although the *in vivo* effects of stress-related hormones on tumor vascularity are currently not known, we are actively studying such effects.

Previous work has supported a relationship between biobehavioral factors, such as social support and distress, and cancer progression (6, 45). However, previous studies provide relatively weak evidence that cellular immune factors account for this relationship (3–6). Although cellular immune mechanisms, such as the cytotoxic activity of NK cells, are important in control of ovarian tumors (46–50), ovarian tumors have a variety of mechanisms to evade immune detection and destruction (51–54). The lack of strong findings in support of mediation by cytotoxic immune factors of a relationship between biobehavioral factors and disease progression suggests that other mechanisms, such as those described above, may underlie these relationships. Taken together with a recent report that the neurotransmitter  $\gamma$ -aminobutyric acid (GABA) inhibits migration of colon cancer cells (55), the present findings suggest new possibilities for direct neurohormonal regulation of tumor cells.

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